

Effect of the source of the plant part and plant growth regulators on the establishment and multiplication of vegetative branches of *Rosmarinus officinalis* L. *in vitro*

Al-Hasan Nassrullah ^{1*}, Sarab Almkhtar ²

¹ College of Agriculture/Department of Horticulture and Landscape/ University of Kerbala/ Kabala/ Iraq; alhasan.a@s.uokerbala.edu.iq. ORCID 0000-0003-1996-7759

² College of Agriculture/Department of Horticulture and Landscape/ University of Kerbala/ Karbala, Iraq; sarab.a@uokerbala.edu.iq. ORCID 0000-0003-0470-7057

* Correspondence: alhasan.a@s.uokerbala.edu.iq; Tel.: 009647706054733

Available from. <http://dx.doi.org/10.21931/RB/2023.08.04.45>

ABSTRACT

This search was carried out in the plant tissue culture laboratory- College of Agriculture - University of Kerbala from 2021 to 2022. The study included the use of *in vitro* technology in the use of plant parts and different combinations in the emergence and multiplication of farms vegetative branches of rosemary, the study was carried out in two stages after performing the sterilization process: the first included the emergence of vegetative farms by planting the growing tops and side nodes on the MS nutrient medium with different concentrations (0, 1, 2, 3 mg.L⁻¹) BA, and the second stage was carried out by cultivating the growths resulting from the nodes grown in the previous stage on the (MS) media, that was prepared with various concentrations (0, 1, 2, 3 mg.L⁻¹) BA and (0, 0.1, 0.2, 0.4mg.L⁻¹) NAA, The results of the study showed the superiority of the apical, as it achieved a response rate of 57.5% compared to the lateral shoots, which recorded a response rate of 37.5%, and the con. of 1 mg.l⁻¹ BA was superior and achieved a response rate of 80% comparison with the neutral treat that achieve a response rate It reached 15%, and the results showed that the same concentration of benzyl adenine at a concentration of 2 mg.l⁻¹ achieved of higher average in the number and length of branches (4.74 branches.plantlet⁻¹, 4.12 cm), while the concentration achieved 3 mg .l⁻¹ of it had the higher rate of leaves number and the Fresh and Dry weight of the branches (15.24 leaves.plantlet⁻¹, 2986 mg and 1823 mg), respectively, comparison with the neutral treat that achieved the lower average, and the concentration 0.2 mg.L⁻¹ NAA exceeded in achieving The highest average number of branches, leaves, fresh & dry weight of branches was (4.16 branches.plantlet⁻¹ and 14.21 leaves.plantlet⁻¹, 2606 mg and 1594 mg) respectively, while the concentration exceeded 0.4 mg.L-1 NAA in achieving the highest rate The length of the branches reached 3.74 cm. All experiments were carried out using CRD (utterly randomized design).

Keywords: explants type ; plant growth regulators ; micro propagation ; rosemary ; tissue culture

INTRODUCTION

The Rosemary plant (*Salvia rosmarinus*), whose scientific name is *Rosmarinus officinalis*, is medically necessary. It is an aromatic perennial plant relearned to the Lamiaceae family. The original home of this plant is southern Europe and the Mediterranean, from which it spread to all parts of the world where it grows wild. Its cultivation spread in most countries, and France, Spain and Tunisia are among the most critical countries

producing the oil extracted from this plant¹. The plant propagates either in the field by seeds. However, it is limited in use during the growing season. It is undesirable, as it is slow because rosemary seeds have low germination levels of about 40%, and vegetatively using Cuttings, the most widely used method for its propagation. However, the ability to root is weak, so researchers tended to multiply it and increase the production of effective compounds by tissue culture². The plant has medicinal and non-medical uses as a food additive, flavoring and spice. It is also used to manufacture aromatherapy, aromatic soap and cosmetics³. Rosemary is one of the plants known for its high nutritional and medicinal value. It has been used since ancient times to enhance memory⁴. The leaves and oil of the plant are often used as spices and flavorings in the food industry because they contain volatile compounds responsible for giving a desirable flavor and as antimicrobials and antioxidants, as plant oil is used. Usually, in the cosmetics and pharmaceutical industries, an aromatic component is included in the manufacture of soap, creams, and moisturizers, and in the perfume industry⁵. The essential oils are found in the leaves and flower tops and are formed from the compounds borneol, camphene, campher and cenol, mainly oleoresin, which is widely used in the preparation of folk medicines and modern pharmaceutical industries, as well as in the manufacture of perfumes, flavors and aromatherapy³, the leaves also contain flavonoids, tannins, rosemarinic acid, diterpenes and rosemaricine⁴. The increasing use and the significant trend in recent years to the utilization of medicinal plants become threatening and a significant risk to plants and natural resources due to their removal and sabotage of the environment. Plants and this technology have significant benefits regarding type, quantity and controlled production without restricting natural factors, such as geographical location, seasonal changes, or environmental stresses. To shorten time and effort, researchers have recently devoted their efforts to using unique methods and technologies that shorten time in breeding and improvement programs and increase the production of secondary compounds by adding some catalysts, whether physical or chemical⁶. Plant growth regulators are organic chemical compounds manufactured naturally or artificially (non-nutrients). They are either stimulants or growth inhibitors that are added in the stages of plant growth and cause a change in its growth and development⁷, Williams⁸ Mention the mechanism of action of plant growth regulators when they are added to plant tissues, added at deficient concentrations and then absorbed from plant tissues by this move to sites to bind to receptors, then a secondary transmission system is activated to stimulate cell activity. PGRsupplemented in the media plays a significant efficacy in determining the desired goal of tissue culture, especially in vitro formation processes⁹. From the above and the importance of the plant from the medical point of view and the efficacy of PGR in the growth and development of the planted plant part, the study aimed to employ the technology of plant tissue culture in the propagation of rosemary plants and study the effect of plant growth regulators added to the nutritional medium with different combinations on the growth and development of the planted part and obtaining greater Sterile, pathogen-free tissue culture.

MATERIALS AND METHODS

Location

The experiment was executed in the plant tissue culture laboratory - Horticulture Department - Agriculture College- University of Karbala from 2021-2022.

2.2. The stage of sterilization of explants and the initiation of plants:-

The explants (shoot tip and buds) For two-year-old plants were laundered with running water several times for an hour, after that in Alcohol 70% for 2 minutes, then with distilled water, then the plant parts were immersed

in 2% NaOCl solution for 15 minutes to be superficially disinfected in sterile conditions. This was followed by washing them with sterile distilled water thrice. To remove the effects of the sterile material, it was prepared, washed in the laboratory and sterilized inside the autoclave. The sterile growing tops and lateral buds were planted on solid {MS (10)} nutrient medium, which was prepared with different concentrations of BA (0, 2, 1, 3 mg.L⁻¹) with 10 replications for each plant part. The cultures were placed in the growth room at controlled growth conditions. The response percentage (percentage of open buds) was taken after 30 days of planting and is calculated according to the following equation: -

Response ratio = (number of buds growing)/(planted to total number of buds)×100%.

Stage of preparation and sterilization of the medium

MS-prepared medium (Murashige, Skoog) containing macro and micronutrients and fortified with vitamins and glycine at a weight of 4.43 gr. L⁻¹ was used throughout the experiment. Sucrose was added—30 gr. L⁻¹, plant growth regulators were added after preparing the base solutions according to the type of experiment, then the pH (Potenz Hydrogen) was adjusted to 5.7 ± 1 using one standard HCL Hydrochloric acid or NaOH. The volume was completed to liter, and agar of the type (Agar-Agar) 7 g per liter was added to the medium for homogenization and dissolution of the agar. Heat the medium using a vibrating heating device until homogeneity and then distribute it in the cultivation tubes (values) up to 10 ml and cover it with the appropriate caps. After preparing the food media and distributing it in the cultivation containers designated for it, it was sterilized with an autoclave at a temperature of 121°C for 15 minutes and kept inside the stratified airflow table until use.

Multiplication stage

Based on the previous stage's results, the growing tops were selected as being the most responsive plant part. The plants were cut at a rate of 2 cm and grown on MS medium prepared with BA (0, 1, 2, 3 mg.L⁻¹) overlapping with NAA (0, 0.1, 0.2, 0.4mg.L⁻¹) at 10 replicates for each concentration. The cultures were kept under the same conditions mentioned above. The study indicators were taken after four weeks, which included the number and length of branches, leaves number, and the Soft and Dry weight of the branches.

Statistical analysis

All experiments were carried out using CRD (utterly randomized design) with factorial experiments with ¹⁰ replications for each treatment. The results were analyzed using the statistical program Genstat, and the averages were compared according to the least significant difference test LSD at the probability level of 0.05 ¹¹.

RESULTS

This class probably swears by subtitles. It should equip a concise and precise description of the results of experiments, their overlap, and the conclusions of experiments that can be carried out.

Effect of explants type and BA concentrations on the Initiation stage

The explants play a vital role in the in vitro cultivation of any plant species due to the determination of the possibility of succession and the rate of branch doubling, and then the physiological condition and sensitivity of plant species to various pollution causes. The results of Table 1. showed the response of the growing tops grown on MS medium in achieving the highest response rate of 57.50% compared to the lateral buds that

recorded a response rate of 37.50%. The con. of 1 mg.l⁻¹ was a significant average of the con., as it gave the highest response average of 80%. In contrast, high levels of BA caused a decrease in the growth rate, which happened at concentrations 2 and 3 mg.l⁻¹, which recorded a response average of 65 and 30% on the relay, while the neutral treat achieved a lower average of 15%. The results confirm that all bilateral interactions significantly affected the response rate. in the case of interaction between the cultivated plant part and BA concentrations, the highest response rate recorded by the growing top at the concentration 2 mg.L⁻¹BA was 90%, as shown in Figure 1, and the lowest response rate recorded by lateral buds in the comparison treatment, which reached 10%.



Figure 1: Response of Shoots tip to concentrations BA to initiation after four weeks of cultivation on MS media.

Explants type	BA Con(mg.l ⁻¹)				mean
	0.0	0.1	0.2	0.3	
Shoots tip	20	90	80	40	57.5
Buds	10	70	50	20	37.5
LSD (0.05)	16.19				8.10
mean	15	80	65	30	
LSD (0.05)	11.45				

Table 1. Effect of plant part type and BA concentrations and their interaction on the response of vegetative parts (%) to initiation after one month of cultivation on (MS) media.

Influence of BA and NAA concentrations and their overlap on the average number of shoots(branches. Plantlet⁻¹).

One of the traits studied is the effect of different BA and NAA concentrations and their interaction on the vegetative multiplication process, which was observed in Table 2. The superiority of BA significantly at the con. of 2 mg.l⁻¹ by giving it the highest average of branches number reached 4.74 branches. Plantlet⁻¹, which did not differ significantly from the con. to 3 mg.l⁻¹, achieved an average of 4.25 branches. plantlet⁻¹, the lowest rate was in the comparison treatment, which amounted to 1.99 branches. plantlet⁻¹. The same table also indicated that NAA was excellent at con. 0.2 mg.l⁻¹, the highest rate was recorded at 4.16 branches. Plantlet⁻¹, which did not differ significantly from the concentration 0.1 mg.l⁻¹, gave an average of 4.00 branches. Plantlet⁻¹, compared to the control treatment, which gave the lowest rate of 3.41 branches. Plant⁻¹. As for the

interaction effect between the concentrations of BA and NAA, their values differed significantly from each other, as the con. of 2 mg.L⁻¹ of BA and the overlap with concentrations 0.1 and 0.2 mg.L⁻¹ of NAA gave the highest average number of branches that reached 5.33 branches. Plantlet⁻¹, as we can see in the figure(2-b), while the medium without growth regulators achieved the lowest response to the number of branches, which reached 1.33 branches. part⁻¹ as we can see in the Figure 2-a. Recorded the highest rate

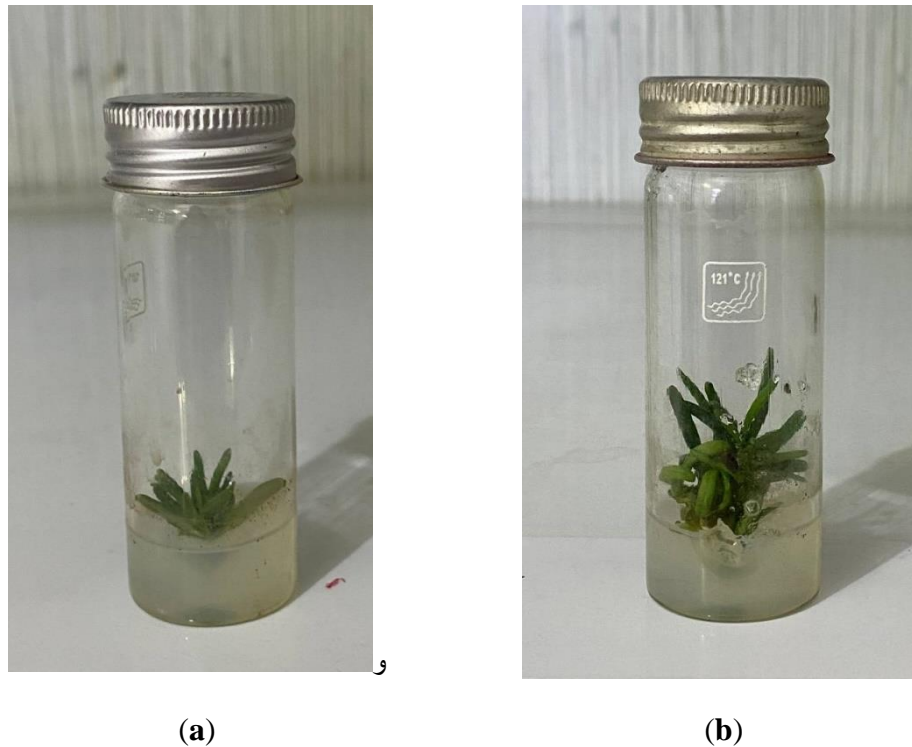


Figure 2: Vegetative branches of rosemary plant after four weeks of cultivation on MS media.: (a) control treatment (Free media without growth regulators), (b) Influence of BA and NAA concentrations and the overlap between them on vegetative branches of rosemary.

BA Con{mg.L ⁻¹ }	NAA Con(mg.L ⁻¹)				mean
	0.0	0.1	0.2	0.4	
0	1.33	1.67	2.33	2.64	1.99
1	4.00	4.33	4.33	3.67	4.08
2	4.33	5.33	5.33	4.00	4.74
3	4.00	4.67	4.67	3.67	4.25
{L.S.D}{(0.05)}	1.12				0.56
mean	3.41	4.00	4.16	3.49	
L.S.D(0.05)	0.56				

Table 2: Influence of BA & NAA concentrations and the overlap between Them on a rate number of vegetative branches after one month of planting on (MS) media.

Influence of BA & NAA concentrations & the overlap between Them on length vegetative branches(cm)

The results of Table 3. show that there was excellence in the average length of rosemary branches when increasing BA concentrations from 1 to 2 mg.l⁻¹ was added to the nutrient medium, which gave a rate of 3.57 and 4.12 cm on the relay. The response decreased with an increase in BA concentration to 3 mg.L⁻¹, which gave the lowest rate of 2.45 cm, while the control treatment gave an average of 3.01 cm.

Regarding the effect of NAA concentrations, the con. 0.4 mg. L⁻¹ was excellent in an average branch length (3.74 cm), while the comparison treatment achieved the lowest rate of 2.42 cm. As for the interaction effect between the concentrations of BA and NAA, it is noted that the higher rate was achieved in the (MS) media, which contains a concentration of 2 mg.L⁻¹ of BA with 0.1 mg.L⁻¹ of NAA, which was 4.63 cm, which was significantly superior to that of NAA. The rest of the treatments, as for the lowest average length of branches, occurred at the empty nutrient medium, which amounted to 1.09 cm.

BA Con{mg.L ⁻¹ }	NAA Con(mg.l ⁻¹)				mean
	0.0	0.1	0.2	0.4	
0	1.09	2.61	4.04	4.29	3.01
1	3.08	3.78	3.91	3.51	3.57
2	3.59	4.63	3.99	4.26	4.12
3	1.93	2.77	2.20	2.91	2.45
{L.S.D}{(0.05)}	0.30				0.15
mean	2.42	3.45	3.54	3.74	
L.S.D(0.05)	0.15				

Table 3: influence of BA & NAA and the overlap between Them on the length rate of vegetative branches (cm) of rosemary after four weeks of cultivation on MS media.

Influence of BA and NAA concentrations the overlap between Them on the leaves number rate (leaves. Plantlet⁻¹)

The results of Table 4. show that the concentration exceeds 3 mg. L⁻¹ significantly recorded the highest average number of leaves, which was 15.24 leaves. Plantlet⁻¹ did not differ significantly from the con. of 2 mg.L⁻¹, as it achieved an average of 15.03 leaves.plantlet⁻¹, while the lowest average number of leaves was in the neutral treatment, which was 9.84 leaves.plantlet⁻¹. Results also showed that the NAA superiority at 0.2 mg L⁻¹ was significant in achieving the highest rate of 14.21 leaves.plantlet⁻¹, compared with the control treatment that gave a rate number of leaves of 12.20 leaves.plantlet⁻¹. As for the overlap effect between the concentrations of BA and NAA, it is noted that the results of BA were excellent at the con. About 2mg. L⁻¹ and with the interaction of the NAA at a con.about 0.2 mg.L⁻¹ in giving, The highest rate of 17.50 leaves.plantlet⁻¹, with comparison treatment which, gave an average of about 8.67 leaves.plantlet⁻¹.

BA Con{mg.L ⁻¹ }	NAA Con(mg.l ⁻¹)				mean
	0.0	0.1	0.2	0.4	
0	8.67	9.00	10.70	11.00	9.84
1	12.60	14.33	12.33	11.33	12.64
2	12.30	16.68	17.50	13.65	15.03
3	15.25	15.75	16.33	13.65	15.24
{L.S.D}{(0.05)}	1.40				0.70
mean	12.20	13.94	14.21	12.40	
L.S.D(0.05)	0.70				

Table 4: influence of BA and NAA and the overlap between them on the rate of leaves number (leaves.plant⁻¹) of rosemary plant after one month of cultivation on MS media.

Influence of BA and NAA concentrations and the overlap between them on the rate fresh weight of shoots (mg)

Regarding the effect of the concentrations of BA and NAA added to the food medium on the characteristic of the fresh weight of the whole vegetable, the statistical analysis data is recorded in Table 5 and indicated that the concentration exceeded 3 mg. L⁻¹ BA significantly recorded the highest fresh weight average of 2986 mg, which did not differ significantly from the con. of 2 mg. L⁻¹, as it gave an average of 2870 mg, whereas the lowest average of fresh weight in the control treatment was 1324 mg. The results also showed the superiority of NAA at a concentration of 0.1 mg.L⁻¹ significantly achieved the highest rate of 2606 mg, which did not differ significantly from the 0.2 mg con. L⁻¹, which achieved a rate of 2553 mg, compared with the control treatment, which achieved the lowest rate of 2037 mg. As for the interaction effect between the concentrations of BA and NAA, it is noted from the results of the table that BA was exceeded at the con. of 2 mg. L⁻¹ and with the interaction with NAA at the con. of 0.2 mg. L⁻¹ achieved the highest rate of 3621 mg, while the comparison treatment achieved less average, amounting to 946 mg.

BA Con{mg.L ⁻¹ }	NAA Con(mg.l ⁻¹)				mean
	0.0	0.1	0.2	0.4	
0	946	1220	1395	1735	1324
1	2082	2884	2044	1952	2241
2	2035	3220	3621	2605	2870
3	3035	3100	3155	2605	2986
{L.S.D}{(0.05)}	294.1				147
mean	2037	2606	2553	2224	
L.S.D(0.05)	147				

Table 5: Influence BA & NAA and the overlap between Them on the rate fresh weight of the shoot (mg) of rosemary after four weeks of cultivation on MS media.

Influence BA & NAA concentrations and their overlap on the dry weight rate of shoots (mg)

The statistical analysis data in Table 6. indicated significant differences in the average Dry Weight characteristic of the vegetative group according to the different concentrations of BA and NAA added to the media, as the con. It exceeded 3 mg. L⁻¹ BA significantly recorded the highest rate of dry weight, about 1823 mg, which did not differ significantly from the con. of 2 mg. L⁻¹, as it achieved an average of 1698 mg, while the lowest dry weight rate when the control treatment was 641 mg. Results also showed the superiority of NAA at a con. of 0.2 mg in the same table. L⁻¹ significantly achieved the highest average of 1594 mg, which did not differ significantly from the 0.1 mg.L⁻¹ achieved an average of 1514 mg, compared to the control treatment, which gave the lowest average of 1112 mg. As for the interaction effect between the concentrations of BA and NAA, it is noted from the table results that BA exceeded the con. 2 mg. L⁻¹ and with the interaction with NAA at the concentration 0.2 mg. L⁻¹ achieved the highest average of 2540 mg, whereas the comparison treatment achieved an average amounting to 330 mg.

BA Con{mg.L ⁻¹ }	NAA Con(mg.l ⁻¹)				mean
	0.0	0.1	0.2	0.4	
0	330	654	750	833	641
1	1061	1240	1018	987	1076
2	1015	2110	2540	1128	1698
3	2040	2055	2070	1128	1823
{L.S.D} _(0.05)	274.2				137.1
mean	1112	1514	1594	1019	
L.S.D _(0.05)	137.1				

Table 6: influence of BA & NAA and the overlap between Them on the Dry Weight rate of the shoot (mg) of rosemary after four weeks of cultivation on MS media.

DISCUSSION

From the preceding, as we can see in Table 1. as a result of the lack of response of the lateral buds to the evolution experiments compared to the developing peaks, the developing peaks were adopted in the evolution experiments and other stages. The reason for the superiority of the ends of the branches may be due to the presence of auxin in the shoot tip more than it is in the single nodes because the shoot tip is the main center for its manufacture in the plant and thus its effect on cell division and elongation is more significant in the ends of the branches ¹² Or, the reason may be attributed to the fact that the developing tops have a number of axillary buds, which have a greater chance of surviving and growing rapidly¹³. Alternatively, the reason is probably on account of physiological factors concerning the hormonal and nutritional content of tissues, which is a determinant of response, whereby nutrients and hormonal substances accumulate in the tissues of the growing apex compared to other parts, or the reason for the superiority of the developing apex may be attributed to the rapid division of its cells because they are unspecialized and undifferentiated cells and in the developmental stages Preliminary¹⁴. These results agree with the findings of several researchers ^{15,16,17} who recorded when using the tops of the branches in extracorporeal propagation. The neighborhood was a huge success. The catalytic action of BA concentrations is attributed to the catalytic action of cytokinin in urging the cultured branch cells to

divide and differentiate, and the differentiation of shoots results in vegetative branches. In addition, many researchers pointed to the role of cytokinein at appropriate concentrations in tissue culture¹⁸. This is consistent with the findings¹⁹ that cytokines have an essential role in the development of cultures and does not agree with the findings²⁰. Also, the decrease in growth rates at high concentrations of benzyl adenine may be due to a disturbance in the vital processes within the plant tissues, which led to an imbalance in the hormonal balance and then a decrease in the growth rates of plant parts²¹.

It was clear from the above-presented results of Tables. 2-6 Generally, the growth regulator BA was superior in the studied traits, which included the number and length of vegetative branches, the number of leaves, and the fresh and dry weight of the vegetative group compared to the comparison treatment. The reason may be attributed to the role played by the balance between the growth regulators used Cytokinines) BA (and Auxins (NAA) in determining the pattern of cellular differentiation and formation of organs outside the body, as the presence of high concentrations of Cytokinines and low Auxins in the food medium leads to the formation of vegetative buds that grow into vegetative branches. Studies indicate that auxin leads to the stimulation of genes that produce cytokines. By controlling their gene expression, gene expression products play an essential role in biological processes such as cell division, chloroplast development, and nutrient metabolism²². These effects (the studied traits) may be due to the catalytic action of cytokines in urging cells to divide and differentiate, resulting in the growth of buds into vegetative branches. Quickly and highly efficient in breaking the apical dominance as it works to reveal and widen the vessels carrying both wood and phloem, prevent the decomposition of chlorophyll, stimulate cell division and increase the production of nucleic acids. The reason may be due to the action of BA on the hormonal balance of the plant tissue in the meristematic areas rich in auxin to cause the required response, which causes a break in the apical dominance and the transfer of nutrients by pushing the plant part to stimulate the growth of vegetative branches in the armpits of the leaves^{23,24,25}, as for the higher levels of them, which cause a decrease in growth rates on account of the disturbance on the vital processes in the tissues as cause of Hormonal Imbalance in them, leading to a decrease in The growth rates of the plant parts. This decrease does not necessarily mean the death of cells, but it is usually the result of an impediment to growth²¹. This is consistent with what was found²⁶ when growing vegetative branches of *Digitalis lanata* by growing the tips of the branches of sterilized seedlings on MS medium prepared with different concentrations of growth regulators BA and TDZ. Results also correspond with the Findings²⁷ when treating the *Digitalis lanata* plant with gamma rays and adenine. The rate of the studied vegetative growth characteristics is also noted from the data of the same tables that BA exceeds the concentration of 2 mg. L⁻¹ in the number and length of multiple branches and the number of leaves. The reason may be attributed to the stimulatory action of cytokinein in urging cells to divide and differentiate, which results in the growth of buds into vegetative branches. Apical dominance and the creation of areas of attraction in the lateral buds stimulate the rapid transfer of nutrients to them, which results in stimulating the growth of buds and, thus, the number of branches. Several theories have been developed to explain this phenomenon, including that the added cytokinein moves from the bottom up through the axillary buds and thus cancels the effect of auxien formed In terminal and inferiorly motile buds, which may accumulate in high concentrations in axillary buds and hinder their growth by inhibiting differentiation in the lateral vascular tissues in these buds. Thus, the role of the cytokinein moving upward will be to affect the process of differentiation of the woody tissues and vascular bundles of the axillary buds to link with their counterparts in the stem and then facilitate the transfer of water and nutrients to these buds and thus stimulate them to grow, develop and form lateral branches²⁸, as for the higher levels of

them, which cause a decrease in growth rates on account of the disturbance on the vital processes in the tissues as a cause of Hormonal Imbalance in them, leading to a decrease in The growth rates of the plant parts. This decrease does not necessarily mean the death of cells, but it is usually the result of an impediment to growth ²¹.

It is also noted from the data of the previous tables that BA is superior to the concentration of 2 mg. Liter⁻¹ in the average fresh and dry weight may be attributed to the fact that this treatment had outperformed the average number and length of branches, which led to an increase in the live mass, which was reflected in the fresh and dry weight of this mass. These results are in agreement with the findings of ^{29,30}.

The highest average fresh and dry weight of the multiplied branches was (106.2 and 5.82) mg, respectively, obtained when the tips of *Digitalis purpurea* branches were grown on MS medium prepared with 2 mg. L⁻¹ BA with 0.2 mg. L⁻¹ IAA. The results also agreed with what was reached ³¹ when conducting the vegetative multiplication experiment of the hawthorn plant, when the concentration was 2 mg.L⁻¹ of BA had the highest rate of fresh and dry weight (345.0 and 102.5) mg, respectively. It also agreed with what was reached ³² in the characteristics of the fresh and dry weight of the vegetative growths of *Catharanthus roseus* planted on MS medium and prepared with 2 mg.L⁻¹ of BA with 0.2 mg.L⁻¹ of NAA gave the highest average the fresh & dry weight (793.0 & 432.3) mg, respectively.

CONCLUSIONS

The selection of a type part of the plant and the addition of plant Growth Regulators in different combinations and concentrations to the MS food medium had a practical and distinct role compared to the comparison treatment in the average of the studied vegetative growth characteristics, which included the number and length of branches, number of leaves, fresh and dry weight of the vegetative total in the farms of vegetative branches of rosemary plants—transplanted in vitro.

Author Contributions: “Conceptualization, Al-Hasan Nassrullah and Sarab Almkhtar; validation, Sarab Almkhtar; formal analysis, Al-Hasan Nassrullah; data curation, Al-Hasan Nassrullah; writing original draft preparation, Sarab Almkhtar; writing review and editing, Al-Hasan Nassrullah; supervision, Sarab Almkhtar; project administration, Al-Hasan Nassrullah; All authors have read and agreed to the published version of the manuscript.”

Funding: "This research received no external funding."

Acknowledgments: Special thanks and gratitude to everyone who contributed to overcoming the difficulties encountered by the experiment, writing the research and publishing it, especially the head of the Department of Horticulture and Landscaping, Dr. Kadum Mohammed Abdullah and Dr. Zaid Khalil, as well as Dr. Zeinab Alywe Mohammed Al-tememe.

Conflicts of Interest: “The authors declare no conflict of interest.”

REFERENCES

1. Peter, V. The secondary metabolism of plants Secondary Defenes compounds. Springer Verlage. New York. **2004**, p.1-10.
2. Leelavathi, D., & Kuppan, N. IN VITRO REGENERATION FROM APICAL BUD EXPLANT OF *ROSMARINUS OFFICINALIS* L. AN IMPORTANT MEDICINAL PLANT. *Banat's Journal of Biotechnology*. **2013**, 4(8), 14

3. Seveitia, M.R., K.M. Abu Amerb and Sena. Pharmacology of rosemary (*Rosmarinus officinalis* L.) and its therapeutic potentials. *Indian Journal of Experimental Biology*. **1999**, 37 (February): 124-131.
4. Chevallier, A. Encyclopedia of Medicinal Plants. Dorling Kindersley. **2001**, 336 pp.
5. Maistor E.L.; S.F. Mota; E.B. L.Ima; B.M. Bernardes and F.C. Goulart. Genotoxicity and mutagenicity of *Rosmarinus officinalis* L. (Labiatae) essential oil in mammalian cells in vivo. *Genet. Mol. Res.* **2010**, 9(4), 2113-2122.
6. Singh, A. ; P. K. Singh. Salicylic acid-induced biochemical changes in cucumber cotyledons. *Indian J. of Agricultural Bioch.* **2008**, 21(1and2), 35-38.
7. Paridaen, A. Investigating the use of plant growth regulators in New Zealand and Australia. Australian University, Crops Competition New Zealand Study Tour Project Report. **2009**.
8. Williams, M.E. Introduction to phytohormones. Doi/ 10. 1105 / tpc. 110. Tt 0310. **2011**
9. Puglisi, S. Use of plant growth regulators to enhance branching of Clematis spp. Master of Science, Department of Horticulture Science, Virginia Polytechnic Institute and State University, Blacksburg. **2002**
10. Skoog, F. and C.O. Miller. Chemical regulation of growth and organ formation on plant tissue cultivated in vitro : In Biological Action of growth substances . 11 th . symp . soc. Exp . Biol. U. k. **1957**, 11:118- 131.
11. Al-Sahoki, Medhat, Waheeb ; Karima Ahmed. Applications in the design and analysis of experiments. Ministry of Higher Education and Scientific Research - Iraq. **1990**.
12. S. F. Al-jughaify, A., Sh. J. Alobaidy, B. Effect Of Osmo-Hardening Seed On K And Na Concentration And Some Growth Properties Of Wheat Under Salt Stress. Anbar Journal Of Agricultural Sciences, 2023; 21(1): 32-43. doi: 10.32649/ajas.2023.179713.
13. Rasool, R.; Ganai B. A.; Kamili ; A.N. Akbar; S, Masood A. *Artemisia amygdalina* (Asteraceae). *a critically endangered plant of Kashmir. pak. J. Bot* **2013**; 45(2):629-634.
14. Hammoud, Ali Khalaf ; Majid, Bayan Hamza. Effect of benzyl adenine and salicylic acid on the growth and production of total alkaloids of Ashwagandha (*Withania Somnifera* L.) ex vivo. *Iraqi Journal of Agricultural Sciences* **2017**, 48(3):700-690.
15. Gantait, S.; N. Mandal; S. Bhattacharya ; P.K. Das. An elite Protocol for accelerated quality-cloning in *Gerbera jamesonii* Bolus cv. sciella . in vitro cellular and Developmental . *Biology-plant* **2010**, 46(6):537-548.
16. Saini , H.K .; M.S. Gill ; M.I.S. Gill . Direct shoot organogenesis and plant regeneration in rough lemon (*Citrus jambhiri* Lush.). *Ind. J. Biotech* **2010**, 9, 419:423.
17. Kumar, R.S; C. Joshi ; K.T. Nailwal. Callus induction and plant regeneration from leaf explant of apple (*pyrus malus* L.) cv. Golden Delicious. *International Journal of Current Microbiology and Applied Sciences* **2016**, 5(2):502-510.
18. Taiz, L. and E. Zeiger . Plant physiology. 5th ed. Sin Auer Associates Inc. Publisher Sunderland, Massachus - AHS. U.S.A. **2010**,
19. Soni, M; M. Thak and M. Modgil. In vitro multiplication of Merton I.793- an apple rootstock suitable for replantation. *Indian Journal of Biotechnology* **2011**, 10(7):362-368.
20. Ohmayed, K. H. .; Sharqi, . M. M. .; Rashid, H. M. . Comparison Of The Physical And Chemical Changes In Local Organic Waste After Cultivation Of The Ganoderma Lucidum Mushroom And Composting By Common Methods. *Journal of Life Science and Applied Research*. 2020, 1, 1-9.

21. Abdulateef, S. M., Aldhanki, Z. T. M. & Rashid, S. A. The influence of different sounds on the feeding behavior of broiler chickens and their impact on blood physiology and conditioning place preference (CPP). *Plant Arch.* 2018.18
22. Schmülling, T. Cytokinins In *Encyclopedia of Biological Chemistry*. Academic Press/Elsevier Science. **2004**
23. Abu Zaid, Al-Shahat Nasr. *Plant hormones and agricultural applications*. Second Edition . Arab House for Publishing and Distribution. Egypt. **2000**
24. Ali, H.H., AL-Rawi, K., Khalaf, Y., Alaaraji, S., Aldahham, B., Awad, M., Al-ani, O., Al-ani, F., Ali, A.T. Serum Caveolin-1 Level is Inversely Associated with Serum Vaspin, Visfatin, and HbA1c in Newly Diagnosed Men with Type-2 Diabetes (2022) *Reports of Biochemistry and Molecular Biology*, 11 (2), pp. 299-309.
25. Schmülling, T. Cytokinins In *Encyclopedia of Biological Chemistry*. Academic Press/Elsevier Science. **2004**
26. Khudai M Y, Abdulateef S M, Mouhammed T Th, Alamili H S. Use of modern geometric design of fish ponds to increase welfare and blood parameters. *Revis Bionatura* 2023;8 (2) 82. <http://dx.doi.org/10.21931/RB/2023.08.02.82>
27. Almukhtar, S. A., Alrubaye, M. A., Elkaaby, E. A., Kadhim, Z. K., : Alkilabi, C. K. Effect of irradiation by gamma rays and the use of benzyl adenine to increase the production of cardiac glycoside compounds from *Digitalis lanata* in vitro. In *IOP Conference Series: Earth and Environmental Science*. **2019**, Vol. 388, No. 1, p. 012068. IOP Publishing.
28. Al-Khafaji, Makki Alwan. *Plant growth regulators - their horticultural applications and uses - Ministry of Higher Education and Scientific Research - College of Agriculture - University of Baghdad*. **2014**
29. F. T. Al-Rawi, Y. T. Abdul-Rahaman , Abdullah I.Noaman , Th. T. Mohammed, S. M Abdulateef, Nadia Jebiril and KI. Mahmud. Role of ascorbic acid and appetite stimulants on a few blood serum biochemical characteristics in pregnant Iraqi ewes under heat stress. Al-Rawi F T, Abdul-Rahaman Y T, Noaman *Revis Bionatura* 2022;7(4) 6. <http://dx.doi.org/10.21931/RB/2022.07.04.6>.
30. Kadhim, Z. K., Al-Shareefi, M. J., Lateef, S. M. Effect of growth regulators on in vitro micropropagation of blue honeysuckle (*Lonicera caerulea* L.). *Research on Crops* **2019**, 20(3).
31. Al-Obaidi, Hashem Kazem Mohammed : Ahmed, Maysa Hamed and Ibrahim, Kazem Mohammed. *CrategusjapanL hawthorn reproduction ex vivo*. *Journal of Al-Mustansiriya Science* **2009**, Volume 20 issue 5 pages1-8.
32. Al-Hujaimi, Ihsan Jali Azbeeb. Using tissue culture technology to produce vincristen and phenplacen in the callus of *Catharanthus roseus* tolerant to salt stress, Master's thesis, College of Agriculture, University of Kufa. **2010**

Received: 26 September 2023 / Accepted: 15 April 2023 / Published: 15 December 2023

Citation: Nassrullah, A.; Almukhtar, S.; The effect of explants type and plant growth regulators on the establishment and multiplication of vegetative branches of *Rosmarinus officinalis* L. *in vitro*. *Revis Bionatura* 2023;8 (4) 45. <http://dx.doi.org/10.21931/RB/2023.08.04.45>

Publisher's Note: Bionatura stays neutral concerning jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2023 by the authors. Submitted for possible open-access publication under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).